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M-a-85 (Revision #16)

December 20, 2018

TO: Director, Office of State Cooperative Programs
Attn: All Staff, Division of Milk Safety

FROM: Milk and Milk Products Branch (HFS-316)

SUBJECT: Beta Lactam and Other Test Methods For Use Under Appendix N And Section 6 Of The *Grade "A" Pasteurized Milk Ordinance* (PMO)

This coded memorandum replaces and rescinds the previous revision of this coded memorandum (M-a-85 (Revision #15), Issued August 29, 2016).

This revision addresses the following changes**:**

Discontinuance of the Neogen BetaStar® Plus Beta-lactam Test
Discontinuance of the Charm® II Cloxacillin Test

Acceptance of the Charm® ROSA® Tetracycline SL Test
Acceptance of the Charm® TRIO Test (screening only for Beta-Lactams, Sulfonamides and Tetracyclines)
Acceptance of the Charm® ROSA® Sulf Test
Acceptance of the Neogen BetaStar® Advanced for Beta-Lactams Test
Acceptance of the Neogen BetaStar® Advanced for Tetracyclines Test

The individual test tables presented in this revision provide data points that were derived from testing at least thirty (30) samples at each concentration for each drug detected.

The attached information is summarized from the evaluation of data submitted by test sponsors. Information related to the protocol used in this evaluation is available from Dr. Philip J. Kijak, FDA's Center for Veterinary Medicine (CVM), (240) 402-6689. Additional information regarding the performance of these tests may be available from the test kit manufacturers.

Label claims for these new approved tests were evaluated for use on raw, commingled cow milk samples as indicated in the tables below. All the information presented in the attached tables is based on the evaluation of the tests using raw commingled cow milk. For tests with a label claim for additional species, the test sponsor provided data to demonstrate that the performance of the test is equivalent to the performance in cow milk. The evaluation protocol did not measure the performance of these tests in the assay of drug residues in other milk matrices, i.e., pasteurized milk or milk taken from individual cows, although claims for such use are made by some of the manufacturers of these tests.

NOTE: Six (6) beta-lactams are widely used in treating disease in lactating dairy cattle and are the most likely to cause a residue in milk if misused. These are Penicillin, Ceftiofur, Cloxacillin, Cephapirin, Amoxicillin, and Ampicillin. While it is preferred that monitoring for beta-lactams includes all of these drugs, at this time, the Agency is recommending that methods be utilized that have been shown to detect at least four (4) of the six (6) beta-lactams identified above.

NOTE: Only one sulfonamide, Sulfadimethoxine, is approved for use in dairy cattle and widely used. The use of unapproved sulfonamides in dairy is prohibited by 21 CFR 530.41 and any extra-label use is illegal. Of unapproved sulfonamides, Sulfamethazine is of primary concern due to the historic record of misuse resulting in residues. The Agency is recommending that methods be utilized that have been shown to detect Sulfamethazine and Sulfadimethoxine.

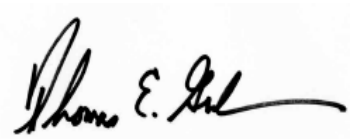
NOTE: The tolerance for tetracycline drugs in milk is based on the sum of three (3) tetracycline drugs. These are Chlortetracycline, Oxytetracycline and Tetracycline HCL. Only Oxytetracycline is approved to be used in treating disease in lactating dairy cattle. Extra-label use of the unapproved tetracyclines and misuse of Oxytetracycline are likely to cause a residue in milk. While it is preferred that monitoring for tetracycline drugs include all three of these drugs, at this time, the Agency is recommending that methods be utilized that have been shown to detect Oxytetracycline and one additional tetracycline drug identified above.

Testing for drug residue(s) in compliance with the provisions of Sections 6 and 7 of the PMO may be accomplished by the use of any accepted Appendix N test for raw milk or an accepted Section 6 test for raw and pasteurized milk.

The National Conference on Interstate Milk Shipments (NCIMS) Executive Board has agreed that future updates to M-a-85 that add, delete or revise these tests will not require a public comment period or follow the protocol established in the Procedures document for the issuance of M-a's.

Copies of this coded memorandum are enclosed for distribution to FDA Milk Specialists, Milk Regulatory Agencies, Laboratory Evaluation Officers and Milk Sanitation Rating Officers. This memorandum should be widely distributed to representatives of the dairy industry, State Veterinarians, State Veterinary and Pharmacy Boards, Veterinarian Professional Organizations and other interested parties and also will be available on the FDA Website at http:// www.fda.gov at a later date.

If you would like an electronic version of this document prior to it being available on the FDA Website, please e-mail your request to Monica.Metz@fda.hhs.gov.



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ATTACHMENT TO M-a-85 (REVISION #16)

Contents

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
BETA LACTAMS

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL	10 ppb	10 ppb	100 ppb[2]	20 ppb	10 ppb	5 ppb
SCREENING TEST						
CHARM® *B. stearothermophilus* TABLET DISK ASSAY[3]	7.5	6.7	ND[4]	11.7	50[5]	3.8
CHARM® II TABLET BETA-LACTAM TEST (COMPETITIVE ASSAY)	7.5	5.7	47	4.2	70[5]	3.0
CHARM® II TABLET BETA-LACTAM TEST (SEQUENTIAL ASSAY)	8.1	6.6	58	4.1	50[5]	3.4
CHARM® II TABLET BETA-LACTAM TEST (QUANTITATIVE ASSAY)[6]	8.1	6.6	58	4.1	50[5]	3.4
CHARM® SL BETA-LACTAM TEST	5.6	8.5	77	13.7	50[5]	3.6
CHARM® 3 SL3 BETA-LACTAM TEST	8.4	8.0	79	20.0	8.6	3.8
CHARM® FLUSLBL FLUNIXIN AND BETA-LACTAM TEST[7]	5.9	6.8	63	13.4	NA[8]	2.0
CHARM® TRIO TEST[9]	3.5[10]	8.8	50	14.5	8.5	2.0
DSM DELVOTEST® P 5 PACK TEST (READER)	4.6	4.0	ND[4]	8.2		2.1
DSM DELVOTEST® P 5 PACK TEST (VISUAL)	4.6	4.0	ND[4]	8.2	NA[8]	2.1
DSM DELVOTEST® P MINI TEST	7.7	5.1	NA[8]	7.0	30[5]	3.1
IDEXX NEW SNAP® BETA-LACTAM TEST	7.3	5.8	12	11.7	50[5]	3.0
NEOGEN BETASTAR® ADVANCED FOR BETA-LACTAMS TEST	9.2	8.6	93	18.1	9.0	4.6

FOOTNOTES:

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following tables and should be considered when selecting drug residue monitoring tests. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory. The data is based on detection in raw, commingled cow milk for the test kits listed.
2. The Ceftiofur tolerance is based on measuring the sum of Ceftiofur and Desfuroylceftiofur related metabolites in milk as Desfuroylceftiofur. The screening test detection concentrations for Ceftiofur were evaluated using milk containing Ceftiofur and Desfuroylceftiofur related metabolites from treated animals.
3. Refer to M-I-01-4, Issued July 2, 2001, for certification requirements to use this visual test
4. ND indicates “Not Detected” at or below tolerance.
5. The 90/95% concentrations were not determined for sensitivities significantly above the tolerance/target testing level.
6. The Charm® II TABLET BETA-LACTAM TEST (QUANTITATIVE ASSAY) may only be used to screen samples. Confirmation will need to be done with another test capable of detecting all 6 beta-lactams.
7. The Charm® FLUSLBL Flunixin and Beta-Lactam Test is a multi-class test. The information listed here is only for the performance of the test kit in detecting beta-lactam drug residues. For information on Flunixin, refer to NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs).
8. NA indicates “Data Not Available
9. The Charm® TRIO Test is a multi-class test and is only approved for screening of milk, i.e., this test may not be used for confirmation. The information listed here is only for the performance of the test kit in detecting beta lactam drug residues. For information on Sulfonamides or Tetracyclines, refer to MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]SULFONAMIDES or TETRACYCLINES.
10. The 90/95 for Amoxicillin is less than 50% of the tolerance and does not meet the requirements of the PMO.

APPROVALS FOR ADDITIONAL MATRICES:

Following approval for raw cow's milk, a manufacturer may submit supplemental data to validate the kit for use in additional milk matrices. Kits approved for additional matrices are listed below by matrix.

Pasteurized Cow's Whole and Skim Milk

CHARM® B. stearothermophilus Tablet Disk Assay
CHARM® II Tablet Beta-Lactam Test (COMPETITIVE ASSAY)
DSM Delvotest® P 5 Pack Test (READER)
DSM Delvotest® P 5 Pack Test (VISUAL)

Pasteurized Cow's Milk fat-free chocolate, whole chocolate, half & half and heavy cream

DSM Delvotest® P 5 Pack Test (VISUAL)

Raw, Commingled Goat Milk

CHARM® B. stearothermophilus TABLET DISK ASSAY
CHARM® II Tablet Beta-Lactam Test (SEQUENTIAL ASSAY)
Charm® SL Beta-Lactam Test (M-I-03-3, Issued 2/25/2003)
DSM Delvotest® P 5 Pack Test (READER)
DSM Delvotest® P 5 Pack Test (VISUAL)
DSM Delvotest® P Mini Test
IDEXX New Snap® Beta-Lactam Test Kit (M-I-13-7, Issued 10/13/2013)

Pasteurized Goat Milk

DSM Delvotest® P 5 Pack (VISUAL)

Raw, Commingled Sheep Milk

Charm® SL Beta-Lactam Test (M-I-09-7, Issued 11/3/2009)

Raw, Commingled Water Buffalo Milk

Charm® SL Beta-Lactam Test (M-I-09-6, Issued 11/16/2009).
DSM Delvotest® P Mini Test (M-I-09-6, Issued 11/16/2009).

Raw, Commingled Camel Milk

IDEXX New Snap® Beta-Lactam Test (M-I-12-13, Issued 10/9/2012).

DRUG CONCENTRATION RESPONSE TABLES FOR APPROVED BETA-LACTAM TESTS

CHARM® B. stearothermophilus TABLET DISK ASSAY

DRUG CONCENTRATION RESPONSE [1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				0
2	0	0	0	0
3				0
4	10	3	0	55
5				100
6	30	67		
8	90	100	0	
10	100	100		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration

CHARM® II TABLET BETA-LACTAM TEST (COMPETITIVE ASSAY)

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					10
2	3	3		30	67
3					97
4	10	43		100	100
5			0		100
6	83	97			
8	100	100		100	
10	100	100	20		
14				100	
20			43	100	
40			100		
60			97		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® II TABLET BETA-LACTAM TEST (SEQUENTIAL ASSAY)

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					0
2	0	0		3	10
3					80
4	20	10		100	100
5			0		100
6	23	83			
8	93	97		100	
10	100	100	0		
14				100	
20			3	100	
40			67		
60			97		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® II TABLET BETA-LACTAM TEST (QUANTITATIVE ASSAY)

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						0
2	0	0		3	0	10
3						80
4	20	10		100	3	100
5			0			100
6	23	83			17	
8	93	97		100	87	
10	100	100	0		100	
14				100		
20			3	100		
40			67			
60			97			
80			100			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® SL BETA-LACTAM TEST

DRUG CONCENTRATION RESPONSE [1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					0
2	3	3			13
3					73
4	70	13		0	100
5			0		100
6	100	83			
8	100	100		50	
10	100	97[4]	0		
12				97	
16				100	
20			0	100	
40			0		
60			23		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites
[4] All statistical models used to calculate 90/95 allow for a single negative result at tolerance

CHARM® 3 SL3 BETA-LACTAM TEST

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						0
2	0	0			0	0
3						13
4	0	0		0	0	97
5						100
6	3	23			13	
8	83	97		0	93	
10	100	100			100	
12				3		
16				83		
20			0	100		
40			0			
60			50			
80			100			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® FLUSLBL FLUNIXIN AND BETA-LACTAM TEST[1]

DRUG CONCENTRATION RESPONSE[2,3]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[4]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					0
2	0	0			20
3					97
4	13	10		0	100
5					100
6	90	43			
8	97	97		3	
10	100	100			
12				67	
16				97	
20			0	100	
40			37		
60			97		
80			100		
100			100		

[1]Beta lactam data only. See separate listing under NSAIDs for flunixin drug concentration response on page 20.
[2] Percent positive
[3] Based on 30 samples at each concentration
[4]Total parent and desfuroylceftiofur related metabolites

CHARM® TRIO TEST[1,2]

DRUG CONCENTRATION RESPONSE[2,3]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[4]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1	3					0
2	60	0			0	93
3	97					100
4	100	3		0	33	100
5						100
6	100	70			80	
8	100	97		30	100	
10	100	100			100	
12				90		
16				100		
20			0	100		
40			27			
50			93			
60			100			
80			100			
100			100			

[1]For screening only, the TRIO TEST is not M-a-85 approved for confirmation of beta-lactam presumptive positive test results. For presumptive positive beta lactam results with the TRIO Test see M-I-96-10 for approved equivalent tests for confirmation and producer trace back.
[2]Beta lactam data only. See separate listing under SULFONAMIDES drug concentration response on page 22 and see separate listing under TETRACYCLINES drug concentration response on page 26.
[2]Percent positive
[3]Based on 30 samples at each concentration
[4]Total parent and desfuroylceftiofur related metabolites

DSM DELVOTEST® P 5 PACK TEST (VISUAL AND READER)

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				3
2	10	7	3	60
3				100
4	100	97	100	100
5				100
6	100	100		
8	100	100	100	
10	100	100		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration

DSM DELVOTEST® P MINI TEST

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				0
2	33	3	0	0
3				100
4	47	70	7	100
5				100
6	93	100		
8	97	100	100	
10	100	97[3]		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]All statistical models used to calculate 90/95 allow for a single negative result at tolerance

IDEXX NEW SNAP® BETA-LACTAM TEST

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					7
2	0	0		0	37
3					93
4	20	37		0	100
5			7		100
6	70	100			
8	100	100		0	
10	100	100	90		
12				100	
20			100	100	
40			100		
60			100		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

NEOGEN BETASTAR® ADVANCED FOR BETA-LACTAMS TEST

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						0
2		0			0	0
3						0
4	0	0			0	90
5						100
6		27			0	
7	27	97				
8	73	100			50	
10	100	100			100	
14				33		
16				73		
18				100		
20				100		
50			0			
60			13			
80			80			
90			97			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

DRUG	FLUNIXIN[2]
TOLERANCE/ TARGET TESTING LEVEL (ppb)	2 ppb
SCREENING TEST	
CHARM® FLUSLBL FLUNIXIN AND BETA-LACTAM TEST	1.9

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each Test in the following Table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.
2. As 5-hydroxyflunixin, the major metabolic form of Flunixin and the chemical marker of Flunixin in milk.

CHARM® FLUSLBL FLUNIXIN AND BETA-LACTAM TEST[1]
DRUG CONCENTRATION RESPONSE[2,3]

DRUG	FLUNIXIN[4]
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**2**
DRUG CONCENTRATION (ppb)	
0.4	30
0.8	70
1.2	97
1.6	97
2.0	100

[1]Flunixin data only. See separate listing under Beta lactams for Beta lactam drug concentration response.
[2] Percent positive
[3] Based on 30 samples at each concentration
[4]As 5-hydroxyflunixin, the major metabolic form of Flunixin and the chemical marker of Flunixin in milk.

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
SULFONAMIDES

DRUG	SULFADIMETHOXINE	SULFAMETHAZINE	SULFATHIAZOLE	SULFADIAZINE
TOLERANCE/ TARGET TESTING LEVEL (ppb)	10 ppb	10 ppb	10 ppb	10 ppb
SCREENING TEST				
CHARM® II SULFA DRUG TEST (COMPETITIVE ASSAY)	4.0	9.4	7.3	4.9
CHARM® ROSA® SULF TEST	7.7	7.8		
CHARM® TRIO TEST[2]	7.6	9.2		

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each Test in the following Table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used.
2. The Charm® TRIO Test is a multi-class test. The information listed here is only for the performance of the test kit in detecting Sulfonamides drug residues. For information on Beta lactams, refer to BETA-LACTAM listing on page 16.

DRUG CONCENTRATION RESPONSE TABLES FOR APPROVED SULFONAMIDE TESTS

CHARM® II SULFA DRUG TEST (COMPETITIVE ASSAY)

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	SULFADIMETHOXINE	SULFAMETHAZINE	SULFATHIAZOLE	SULFADIAZINE
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**10**	**10**	**10**	**10**
DRUG CONCENTRATION (ppb)				
2	97	7	0	40
4	100	80	57	100
6	100	97	100	100
8	100	100	100	100
10	100	100	100	97[3]

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]All statistical models used to calculate 90/95 allow for a single negative result at tolerance

CHARM® ROSA® SULF TEST

DRUG CONCENTRATION RESPONSE[1,2]

Drug	Sulfamethazine	Sulfadimethoxine
Tolerance/Target Level (ppb)	**10**	**10**
Drug Concentration (ppb)		
2	0	3
4	50	67
6	83	83
8	100	93
10	100	97

[1]Percent positive
[2]Based on 30 samples at each concentration

CHARM® TRIO TEST[1,2]

DRUG CONCENTRATION RESPONSE[2,3]

Drug	Sulfamethazine	Sulfadimethoxine
Tolerance/Target Level (ppb)	**10**	**10**
Drug Concentration (ppb)		
1		13
2	3	37
4	17	80
6	70	93
8	90	100
10	100	97

[1]For screening only, the TRIO TEST is not M-a-85 approved for confirmation of sulfonamide presumptive positive test results. For presumptive positive sulfonamide results with the TRIO Test see M-I-96-10 for approved equivalent tests for confirmation and producer trace back.
[2]Sulfonamide data only. See separate listing under BETA LACTAMS drug concentration response on page 16 and see separate listing under TETRACYCLINES drug concentration response on page 26.
[2]Percent positive
[3]Based on 30 samples at each concentration

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1,2]
TETRACYCLINES

DRUG	CHLORTETRACYCLINE	OXYTETRACYCLINE	TETRACYCLINE
TOLERANCE (ppb)	**300**[2]	**300**[2]	**300**[2]
DRUG CONCENTRATION (ppb)			
CHARM® II TETRACYCLINE DRUG TEST (COMPETITIVE ASSAY)	257	119	67
Charm® ROSA® Tetracycline SL Test (Dilution Protocol)	292	243	74
CHARM® TRIO TEST[4]	34	53	42
Neogen BetaStar® Advanced For Tetracyclines Test	254	190	245

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each Test in the following Table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used.
2. The tolerance is established as the sum of residues of the tetracyclines including chlortetracycline, oxytetracycline, and tetracycline
3. Data demonstrated test will detect chlortetracycline at 600 ppb. As the test will not detect chlortetracycline at or below 300 ppb tolerance, 90/95% concentration was not calculated.
4. The Charm® TRIO TEST detects tetracyclines at concentrations less than the approved PMO minimum levels and cannot be used for confirmation. Any TRIO TEST tetracycline positive tests must be confirmed using an M-a-85 approved test that detects all three tetracycline drugs at or below Tolerance.

DRUG CONCENTRATION RESPONSE TABLES FOR APPROVED TETRACYCLINE TESTS

CHARM® II TETRACYCLINE DRUG TEST (COMPETITIVE ASSAY)

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**300 ppb[3]**	**300 ppb[3]**	**300 ppb[3]**
DRUG CONCENTRATION (ppb)			
20			0
30			7
40			37
60		13	93
70		37	
90	17		
100		87	
120	20		
150		100	
160	77		
230	93		
300	97[4]	100	100

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]The tolerance is established as the sum of residues of the tetracyclines including chlortetracycline, oxytetracycline, and tetracycline
[4]All standard statistical models used to calculate 90/95 allow for a single negative result at tolerance

CHARM® ROSA® TETRACYCLINE SL (DILUTION CONFIRMATION) TEST

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**300 ppb[3]**	**300 ppb[3]**	**300 ppb[3]**
DRUG CONCENTRATION (ppb)			
20			0
40			10
60	3	3	70
80			100
120	27	37	
180	23	77	
240	93	90	
290	97		
300	100	100	100

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]The tolerance is established as the sum of residues of the tetracyclines including chlortetracycline, oxytetracycline, and tetracycline

CHARM® TRIO TEST[1,2]

DRUG CONCENTRATION RESPONSE[3,4]

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**300 ppb[5]**	**300 ppb[5]**	**300 ppb[5]**
DRUG CONCENTRATION (ppb)	**10**		
10			
15			60
20	63		97
30		7	100
40	83	30	
50		63	
60	97	93	100
80		100	
100	100	100	100
120	97	100	100
180	100	100	100
240	100	100	100
300	100	100	100

[1]For screening only, the TRIO TEST is not M-a-85 approved for completion of presumptive testing of initial tetracycline test results. After an initial positive test for tetracyclines using the TRIO TEST users MUST switch to the Charm ROSA Tetracycline SL Test (dilution protocol) to complete presumptive testing. For presumptive positive tetracycline results with the Charm ROSA Tetracycline SL Test (dilution protocol) complete confirmation and producer trace back testing using the Charm ROSA Tetracycline SL Test (dilution protocol) or see M-I-96-10 for other approved equivalent tests.

[2] See separate listing under BETA LACTAM drug concentration response on page 16 and see separate listing under SULFONAMIDES drug concentration response on page 22

[3]Percent positive

[4]Based on 30 samples at each concentration

[5]The tolerance is established as the sum of residues of the tetracyclines including chlortetracycline, oxytetracycline, and tetracycline

NEOGEN BETASTAR® ADVANCED FOR TETRACYCLINES TEST

DRUG CONCENTRATION RESPONSE[1,2]

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/ TARGET TESTING LEVEL (ppb)	**300 ppb[3]**	**300 ppb[3]**	**300 ppb[3]**
DRUG CONCENTRATION (ppb)			
50	0	0	0
100	0	0	0
150	0	40	17
200	13	100	93
250	87	100	100
300	100	100	100

[1]Percent positive
[2]Based on 90 samples at each concentration (30 each from three lots)
[3]The tolerance is established as the sum of residues of the tetracyclines including chlortetracycline, oxytetracycline, and tetracycline